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Female ornaments in the Pied Flycatcher *Ficedula hypoleuca*: associations with age, health and reproductive success

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Female ornamentation has received little attention in studies of sexual selection. Traditionally, female ornaments have been explained as a genetically correlated response to selection in males. However, recent findings suggest that female ornaments may be adaptive. Southern populations of Pied Flycatchers *Ficedula hypoleuca* are suited for studies of female ornamentation because, in addition to the white wing patch, some females also express the white forehead patch characteristic of males. We thus addressed the associations of these two ornaments with female age and with some health and breeding parameters in a Spanish population of Pied Flycatchers. Female ornament expression was not associated with haemoparasite prevalences, clutch size or parental provisioning effort. However, females expressing the white forehead patch raised more fledglings, and females with larger wing patches bred earlier, had higher number of hatchlings and showed increased levels of total serum immunoglobulins. Thus, these two unrelated epigamic ornaments may indicate some aspects of female quality. Further experimental studies could test the possibility that these plumage traits might function as signals to the males or might be used during female–female aggressive encounters in competition for nest-sites and mates.

Darwin (1871) defined sexual selection as the process driving the evolution of traits that enhance mating success through competition between individuals of the chosen sex, usually males, and through mate choice by individuals of the choosy sex. In most examples he found, females were the less-ornamented of the two sexes. Except for the cases of sex-role reversed species, Darwin (1871) considered it less plausible to attribute female ornamentation to sexual selection than to a by-product of the trait selected for in males, an explanation which is nowadays known as the genetic correlation hypothesis. The correlated selective responses between the sexes lead to the assumption that male and female phenotypes cannot evolve independently (Lande 1980) and some studies have supported it (Muma & Weatherhead 1989, Hill 1993, Cuervo *et al.* 1996, Rohde *et al.* 1999). However, other authors have come to a different conclusion. Female showiness may have an adaptive meaning, being favoured, for instance, through male mating preferences (Amundsen *et al.* 1997, Jones & Hunter 1993, Torres & Velando 2005) or through intrasexual competition for limiting resources (West-Eberhard 1979, Bleiweiss 1985).

If there is a functional explanation for female ornaments, ornamented females might be signalling some aspects of their phenotypic quality to conspecifics. Indeed, there is evidence that ornamental female traits might indicate immunocompetence (Hanssen *et al.* 2006), age-related quality (Komdeur *et al.* 2005), higher reproductive performance (Møller 1993), low levels of blood parasite infection (Potti & Merino 1996), superior condition (Johnsen *et al.* 1996, Velando *et al.* 2001, Pilastro *et al.* 2003, Jawor *et al.* 2005) or higher provisioning effort to offspring (Linville *et al.* 1998). However, the functional interpretation of female ornamentation is far from clear because, relative to the number of studies of male ornaments, very little is known about

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the function and evolution of decorative traits in females (see Amundsen 2000 for a review on birds).

In the Pied Flycatcher *Ficedula hypoleuca*, males and females differ in their plumage colour, but also in the expression of a white forehead patch. This trait is very conspicuous in males after their first pre-breeding moult (Lundberg & Alatalo 1992), while not present in females according to Svensson (1984). However, in southern populations, some females exhibit a distinctive forehead patch, in some cases as large as the largest patch in males (Potti 1993, J. Morales pers. obs.). The expression of this trait in males has been reported to be preferred by females during mate choice (Potti & Montalvo 1991a), to affect female reproductive allocation (Osorno *et al*. 2006) and to be traded-off against immune capacity (Kilpimaa *et al*. 2004), indicating that it might be costly to maintain. Its expression in females has been reported to be age-dependent (Potti 1993), being mostly absent in young females, and with the size of the patch early in her life influenced by the size of the father's patch. Interestingly, Potti and Merino (1996) showed that its presence was related to decreased levels of trypanosome infection, thus giving support to the Hamilton and Zuk (1982) hypothesis on parasite-driven sexual selection, and that there was assortative mating according to this trait. Nevertheless, in the same population, Potti (1993) did not find associations between the expression of female forehead patch and reproductive parameters.

Ficedula flycatchers also show a white wing patch at the base of the outer vanes of primaries, expressed to a larger extent in populations of central Spain than in northern populations (Curio 1960). In male Collared Flycatchers *Ficedula albicollis*, this trait is displayed during courtship flights (Gustafsson *et al*. 1994), has been shown to be sexually selected (Sheldon & Ellegren 1999) and may act as a condition-dependent signal of genetic quality, being highly repeatable, heritable and a predictor of adult survivorship (Török *et al*. 2003). However, to our knowledge, the potential signalling role of the white wing patch in *Ficedula* flycatcher females has not yet been explored. Wear of de-pigmented areas might be costly, as the lack of melanin weakens the feathers, which suffer from abrasion (Barrowclough & Sibley 1980, Bonser 1995) and a higher probability of breakage and attack by feather-degrading bacteria (Burtt & Ichida 2004) or lice (Kose & Møller 1999). De-pigmentation on wing feathers and on the primaries in particular might be especially costly owing to its possible negative impact on flight performance (Török *et al*. 2003). Therefore, if there is a cost for the bearer, it is not credible that females might express white patches just as a genetically correlated response to the trait in males.

Our first aim in this study was to examine the variation of forehead and wing patch expression within individual females between years, based, respectively, on 8 and 2 years of data from a southern population of Pied Flycatchers. If these ornaments serve as indicators of female quality, we expect them to be positively associated with condition and immunocompetence and negatively with haemoparasite prevalences. We thus assessed the levels of total circulating immunoglobulins (IgY) in the serum. IgY is the dominant circulating antibody in birds and is produced by B-lymphocytes, which are responsible for life-long immunological memory of humoral responses (Warr *et al*. 1995). Higher IgY levels can be regarded as a greater allocation of these responses, either as a phenotypic response to the antigenic environment or as a genetic response to selection (Apanius & Nisbet 2006). Total IgY levels in Pied Flycatcher females of our population remain constant throughout the breeding season, respond to parasitism and stress, and are positively associated with phytohaemagglutinin (PHA) response, when controlling for these other factors (Morales *et al*. 2004). We therefore assume that high levels of IgY are a sign of a good immune capacity (Morales *et al*. 2004). To control for the effects of physiological stress on IgY levels, we measured levels of the stress protein HSP70 in the blood (Morales *et al*. 2004). We also predict that the most ornamented females may invest more in reproduction and may also influence males' investment decisions, inducing them to work harder during offspring provisioning. Finally, we predict that assortative mating might occur, as found by Potti and Merino (1996) for the forehead patch in males in another population of Pied Flycatchers.

MATERIALS AND METHODS

General methods

The Pied Flycatcher is a small (12–13 g), migratory, hole-nesting passerine of European woodlands (Lundberg & Alatalo 1992). The study was carried out during the breeding seasons of 1997–2004 in a population of Pied Flycatchers breeding in Valsaín, central Spain, which has been the subject of a long-term study since 1991 (Sanz *et al*. 2003). Egg-laying in the population under study typically begins in late

May and clutch sizes in our population range from four to seven eggs with a mode of six eggs. On first capture, females were classified as of one or more years of age according to Svensson (1984) and Jenni and Winkler (1994). For most females exact age was known as they were ringed as nestlings in the study area. We assumed an age of 2 years for all recruited adult females that were not raised in the study area (Sanz & Moreno 2000). In reality, some adult females were older than this. However, the allocation of a minimum estimated age ensured that we were able to distinguish forehead patch expression probability in first-year and adult birds using the largest possible sample of birds. With only 2 years of data for female wing patch, we were unable to check for the longitudinal variation of this trait at different age intervals.

In 1998 and 2002, females were captured at the nest on the day after clutch completion and, in 2004, on the day after hatching. These phases are considered as initial breeding stages. The birds were blood-sampled and weighed with a Pesola spring balance (accuracy of 0.25 g). In all breeding seasons, adults were captured at the nest with nest-box traps on day 11–12 of nestling age (hatching day = day 0). Except for the years 1997 and 2001, females were also blood-sampled at this stage. They were weighed and their tarsus length was measured with digital callipers (calliper accuracy 0.01 mm). As a measure of body condition we used mass divided by the cube of tarsus length. When nestlings were 10–12 days old, parental provisioning rates were monitored as the number of feeding trips performed by both parents. The entrance of the nest-boxes was recorded for 1 h with a video camera placed 5–10 m away from the nest-box, between 09:00 and 18:00 h. Provisioning rates of the adults were unrelated to time of day (all $P > 0.2$).

Ornaments in males and females

In all the breeding seasons, the white forehead patch in females was recorded as present or absent, while in males the length and breadth of the forehead patch was measured with callipers to the nearest 0.01 mm and its size (mm^2) was calculated as the area of a rectangle. A few females showed several scattered white feathers above the bill, which do not form an obvious patch, but are clearly distinctive from females completely lacking a white forehead patch. We scored these cases as presence of white forehead patch.

In 2003 and 2004, following Török *et al*. (2003), we measured using callipers (accuracy of 0.01 mm) the white wing patch size on the right wing of females and males as the sum of lengths of white from the tip of primary coverts on the outer web of the third to seventh primaries. Török *et al*. (2003) measured the length of the white area in primaries four to eight in the Collared Flycatcher, but instead we obtained the lengths of white in primaries three to seven in the Pied Flycatcher, as in most cases the eighth primary lacks a de-pigmented area (J. Morales pers. obs.; see also Svensson 1984) and because, according to Curio (1960), birds in central Spain have more white on the wing, often from the fourth and sometimes from the third primary. We obtained two consecutive measures of this trait from 18 females. The measurement of female wing patch size was highly repeatable ($r = 0.96$, $F_{17,18} = 46.51$, $P < 0.001$; Lessells & Boag 1987).

Blood sampling and haemoparasite detection

A blood sample (80 μL) was collected from the brachial vein of females in order to detect the presence of the two most common haemoparasites, *Trypanosoma* and *Haemoproteus*, both at early breeding stages (in 1998, 2002 and 2004) and during the final phase of the reproductive period (in all the breeding seasons except 1997 and 2001). After a blood smear was obtained, the remaining blood sample was centrifuged (2000 ***g***, 5 min; Labnet, catalogue no. 1201-220V, Woodbridge, NJ, USA). Cellular and plasma components were separated and frozen on the same day for later analyses. The blood smear was air-dried, fixed in absolute ethanol and stained with Giemsa-stain (1 : 10 v/v) for 45 min. One half of each smear was scanned under 200× magnification in search of large extra-erythrocytic parasites, such as *Trypanosoma*. In the other half, 50 fields were scanned using 1000× magnification in search of intra-erythrocytic parasites, such as *Haemoproteus* (Merino *et al*. 1997).

Determination of the levels of immunoglobulins and physiological stress

The level of total circulating immunoglobulins (IgY) of females was estimated following the protocol of Martínez *et al*. (2003), at early breeding stages (in 2002 and 2004) and at day 11–12 of the nestling period (in 2002 and 2003). As IgY levels may be

affected by presence/absence of *Haemoproteus* and levels of physiological stress (Morales *et al*. 2004), we have corrected for these two parameters in order to explore the association between female ornamentation and IgY levels. We used absorbance as the final value of IgY concentration or level.

We estimated the levels of the heat-shock protein HSP70 as a measure of physiological stress. The heat-shock proteins are a set of highly evolutionarily conserved molecules that facilitate protein folding and assembly and maintain cellular homeostasis under a wide variety of environmental stressors (Sørensen *et al*. 2003). We determined the levels of HSP70 by means of a Western blot. For details of the protocol see Moreno *et al*. (2002) and Tomás *et al*. (2004).

Statistical analyses

With the exception of analyses of longitudinal variation of female ornaments with age, females captured in different years were included only once in data analyses, in order to avoid pseudoreplication. For this purpose, we have randomly selected one single case for those females captured in more than one year. In 2004 only one female showed infection by trypanosomes at any breeding stage. We thus excluded this year from the analyses including trypanosome data and sample sizes are therefore smaller than those shown for *Haemoproteus*.

Reproductive and physiological variables were entered as dependent variables in the Statistica GLM module (2001), in order to associate them with female ornament expression. In all these analyses we corrected for female minimum age and study year. The model was a three-way ANOVA for the analyses including the forehead patch and an ANCOVA for those including the wing patch. We have assumed normal errors. Haemoparasite prevalences (i.e. presence or absence of haemoparasites) were entered as dependent variables in logistic regressions, in the Statistica GLZ module (2002), in order to associate them with female ornament expression. In these analyses we could not correct for female minimum age and year, as there was not enough variance to check simultaneously for the effect of various categorical factors. Instead, we associated female minimum age with haemoparasite prevalences in separate GLZs.

RESULTS

Female wing patch size was not associated with the presence of a forehead patch (forehead patch: $F_{1,74} = 0.08$, $P = 0.78$; year: $F_{1,74} = 5.68$, $P = 0.020$; age: $F_{5,74} = 0.27$, $P = 0.93$).

Age-correlated changes in female ornaments

A logistic regression with minimum age category and year as factors revealed that the presence of a forehead patch among individuals within years was affected by age, older females being more likely to present it than younger females (age: W (Wald statistic) = 37.0, $P < 0.001$; year: $W = 14.60$, $P = 0.04$; $n = 320$). The proportion of females expressing a forehead patch at each age class was: 11.9% for 1-year-old females, 41.5% for 2-year-old females, 63% for 3-year-old females, 47.4% for 4-year-old females and 71.4% for females older than 4 years. Longitudinal data from individuals that were recaptured in two or more years were obtained from 90 females. In 18 females the forehead patch was absent at first capture but present in the following one, in 33 it was always present, in 33 it was always absent, and only in six cases was it present at first capture and absent in the following one (average of 1.6 years between first and last capture). The frequency of females that did not present the forehead patch at last capture was significantly higher than expected by the hypothesis of presenting the patch as a consequence of ageing (χ^2 test: 19.93, $P < 0.001$). Table 1 presents the direction of change in forehead

Table 1. Direction of change in female forehead patch in different age intervals. The same individual can be represented in different age intervals.

	Forehead patch			
Age interval group (years)	absent – absent	absent – present	present – present	present – absent
1–2	7	6	1	
1–4			1	
2–3	17	12	15	1
2–4	3	2	5	
2–5		1		
2–7				1
3–4	9	5	11	2
3–5	1	1	2	
3–6		1	2	
3–7	1			
4–5	1	3	2	1
4–6	1	1		
> 5		2	3	1
Total	40	33	42	6

Table 2. Associations of the expression of forehead and wing patches with haemoparasite prevalences at initial and final breeding stages.

Haemoparasite prevalences	Forehead patch			Wing patch		
	W	*P*	*n*	*W*	*P*	*n*
Haemoproteus initial	0.14	0.71	91	0.36	0.55	39
Haemoproteus final	1.15	0.28	169	0.09	0.77	70
Trypanosoma initial*	2.62	0.11	39	–	–	–
Trypanosoma final	0.04	0.84	117	2.92	0.087	31

*In 2004, only one female was infected by trypanosomes. Trypanosome prevalence was thus not analysed in this year.

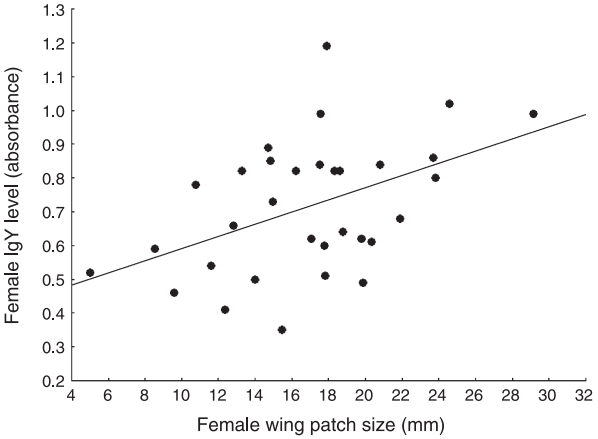

Figure 1. Association of the level of total immunoglobulins (IgY) at the end of the nestling period in 2003 with the size of female wing patch ($r = 0.47$, $F_{1,30} = 8.30$, $P = 0.007$).

patch shown by recaptured females in different age intervals. Because we were interested in the effect of age on the expression of ornaments, the same individual can be represented in different age intervals, if captured more than once. One-third of 121 records never expressed the white forehead patch, independently of their age category (Table 1). Thus, the white forehead patch is not an inevitable consequence of ageing.

Wing patch size was affected by year but not by age (age: $F_{5,75} = 0.26$, $P = 0.93$; year: $F_{1,75} = 5.87$, $P = 0.018$). We were unable to obtain any conclusion based on within-individual changes of wing patch size in different age intervals, as only 20 females were recaptured from 2003 to 2004. Of these 20 females, 14 increased the wing patch from one year to the next, while only six reduced it (two-tailed binomial test: $P = 0.048$). Female wing patch size was repeatable within individuals between two consecutive years ($r = 0.46$, $F_{19,20} = 2.68$, $P = 0.02$).

Female ornamentation and physiological variables

Contrary to expectations, the expression of a forehead patch was not associated with haemoparasite prevalence (Table 2). As we could not correct for female age in these analyses, we analysed haemoparasite prevalence in relation to minimum age in each year and found no association (all $P > 0.2$). Forehead patch expression was not associated with condition or IgY levels, when correcting for year and age (all $P > 0.10$). It was not associated either with the change in IgY levels throughout the reproductive period in 2002 (all $P > 0.4$).

Wing patch size was not associated with haemoparasite prevalence (Table 2). It was not associated with either condition or the initial levels of total IgY, when correcting for year and age (all $P > 0.16$). However, wing patch size was positively associated with the final levels of total IgY (Fig. 1; wing patch size: $F_{1,25} = 7.63$, $P = 0.011$; age: $F_{5,25} = 0.41$, $P = 0.84$). This association remained significant even after correcting for the effects of the presence/absence of *Haemoproteus* and level of HSP70. However, these two parameters were not related to the IgY levels at this stage (both $P > 0.12$), as predicted by the results of a previous study (Morales *et al.* 2004).

Reproductive performance

Females expressing a forehead patch did not breed earlier and did not lay larger clutches (Table 3). However, they raised more fledglings than females without a forehead patch (Table 3; mean ± se: 4.7 ± 0.1 and 4.1 ± 0.1, respectively). The expression of the forehead patch was not related to parental provisioning rates (Table 3).

Females exhibiting larger wing patches laid eggs earlier, but did not lay larger clutches (Table 4). Although they had a higher number of hatchlings, they did not raise more fledglings than females with smaller wing patches (Table 4). This was not due to a higher mortality of nestlings for females with larger wing patches (all $P > 0.05$, $n = 65$ nests). The size of the female wing patch was not related to parental provisioning rates (Table 4).

Table 3. Associations of reproductive parameters (as dependent variables) with the expression of female forehead patch, controlling for year and age.

Reproductive parameter	r^2	Forehead patch			Year			Age		
		F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Laying date	0.10	2.21	1, 306	0.14	1.88	7, 306	**0.073**	3.96	5, 306	**0.002**
Clutch size*	0.37	0.10	1, 305	0.77	3.63	7, 305	**< 0.001**	0.52	5, 305	0.76
Number hatched	0.04	2.07	1, 303	0.15	2.88	7, 303	**0.006**	0.38	5, 303	0.86
Number fledged	0.11	4.82	1, 289	**0.029**	3.39	7, 289	**0.002**	1.75	5, 289	0.12
Female provisioning†	0.22	0.55	1, 148	0.46	5.67	7, 148	**< 0.001**	0.50	5, 148	0.79
Male provisioning†	0.20	0.58	1, 148	0.45	4.04	7, 148	**< 0.001**	1.66	5, 148	0.15

*The effect of laying date was corrected for: $F_{1,305} = 144.47$, ***P* < 0.001**.
†The effect of brood size was corrected for: $F_{1,148} = 25.44$, ***P* < 0.001** and $F_{1,148} = 24.42$, ***P* < 0.001**, respectively.

Table 4. Associations between reproductive parameters (as dependent variables) and female wing patch size, controlling for year and age. The adjusted r^2 is presented for those models where wing patch size showed a significant association with the dependent variable at $P < 0.05$.

Reproductive parameter	r^2	Wing patch			Year			Age		
		F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Laying date	0.50	7.53	1, 74	**0.008**	58.49	1, 74	**< 0.001**	1.72	5, 74	0.14
Clutch size*		1.47	1, 73	0.23	1.35	1, 73	0.25	0.52	5, 73	0.76
Number hatched	0.08	6.16	1, 74	**0.015**	6.11	1, 74	**0.016**	0.25	5, 74	0.62
Number fledged		0.004	1, 64	0.95	0.14	1, 64	0.71	0.88	5, 64	0.50
Female provisioning†		0.02	1, 55	0.88	2.73	1, 55	0.10	0.82	5, 55	0.54
Male provisioning†		1.55	1, 55	0.22	1.39	1, 55	0.24	0.10	5, 55	0.99

*The effect of laying date was corrected for: $F_{1,73} = 12.13$, ***P* < 0.001**.
†The effect of brood size was corrected for: $F_{1,55} = 10.68$, ***P* = 0.002** and $F_{1,55} = 7.69$, ***P* = 0.008**, respectively.

Male traits and female ornamentation

In breeding pairs, the expression of female forehead patch was not associated with male forehead patch size, when taking into account male minimum age, year and female age (male forehead patch size: $W = 1.18$, $P = 0.28$; year: $W = 12.92$, $P = 0.074$; female age: $W = 29.23$, $P < 0.001$; male age: $W = 3.74$, $P = 0.59$). We performed the previous GLZ model to associate forehead patch expression between mates within two different age categories: 1-year-old females or females older than 1 year. But forehead patch expression was not associated with male forehead patch size within the two age categories (all $P > 0.1$).

Female wing patch size (as the dependent variable in a GLM) was not associated with male wing patch size (male wing patch size: $F_{1,51} = 2.65$, $P = 0.11$; female age: $F_{5,51} = 0.24$, $P = 0.94$; male age: $F_{5,51} = 0.31$, $P = 0.90$; year: $F_{1,51} = 3.47$, $P = 0.068$). We performed the previous GLM model within two different female age categories: 1-year-old females and females older than 1 year. For 1-year-old females that were paired with 1-year-old males, there was a marginally positive association between wing patch size of mates (male wing patch size: $F_{1,19} = 4.39$, $P = 0.050$; year: $F_{1,19} = 0.03$, $P = 0.86$). The same trend was found for females older than 1 year that were paired with 1-year-old males ($F_{1,52} = 3.61$, $P = 0.063$; year: $F_{1,52} = 3.74$, $P = 0.060$). There was no association for females that were paired with old males (all $P > 0.1$).

DISCUSSION

Age-correlated changes in female ornaments

The expression of a white forehead patch in Spanish Pied Flycatcher females has been reported as a

reliable sign of ageing (Karlsson *et al.* 1986, Potti 1993). In accordance with this assumption, cross-sectional analyses in the present study showed that the forehead patch is an age-dependent trait. However, although most yearling females did not express a forehead patch, longitudinal changes revealed that a high proportion of adult females (see for instance the 2–3-year and 3–4-year age intervals in Table 1) never expressed it or expressed it for the first time at an advanced age. In fact, Potti (1993) showed that of 13 females scored in consecutive years, more than half never expressed a forehead patch. Therefore, we may conclude that the forehead patch in Pied Flycatcher females in our population might not function as a fairly reliable cue of its bearer's 'breeding experience', and that this character should be used with caution in age determination.

Wing patch size was not associated with female minimum age in cross-sectional analyses. However, to check for longitudinal changes more years of data are needed.

Female quality

We found no evidence for associations of female forehead or wing patch expression with body condition. It is likely that different developmental constraints are associated with different types of ornaments. For instance, carotenoid-based plumage colorations seem to be more dependent on physical condition than melanin-based coloration (Badyaev & Hill 2000). However, de-pigmented areas, such as the forehead and wing patches in *Ficedula* flycatchers, are likely to be less resistant to feather-degrading bacteria (Burtt & Ichida 2004) and abrasion (Barrowclough & Sibley 1980, Bonser 1995), and to suffer breakage and attack by lice (Kose & Møller 1999). In addition, white patches may reveal the quality of their bearer through the regularity (or immaculateness) of its borders (Ferns & Hinsley 2004). The borders between different blocks of colour may be particularly susceptible to damage, as a consequence of attacks by other individuals (Ferns & Hinsley 2004). Therefore, some associated costs not related directly to physical condition might be expected.

We also explored whether the expression of both ornaments was restricted to females with reduced levels of haemoparasite infection, as found by Potti and Merino (1996) for the female forehead patch. Contrary to our expectation, neither forehead patch expression nor wing patch size was associated with haemoparasite prevalences. It is possible that the importance of vectors and thus of trypanosome infection may change between years with varying environmental conditions. Indeed, in 2004 we found no females infected by trypanosomes. Consequently, if the ornament signals trypanosome resistance, its content would be unreliable in some years. Another possible explanation for these contrasting findings is that study areas differ in ways that affect the relative importance of different parasites as influences on the expression of female ornaments.

There was a positive association between wing patch size and total immunoglobulin levels (IgY) in the blood, as measured at the end of the nestling period in 2003. This was true even after correcting for the effect of *Haemoproteus* infection and physiological stress levels, as these two parameters may affect IgY levels (Morales *et al.* 2004). Consequently, the size of the white wing patch could be associated with immunocompetence through unknown mechanisms and could be used by females as an indicator of high immune capacity. This result concurs with a previous study in which the expression of white wing plumage was positively associated with immunocompetence in female Common Eiders *Somateria mollissima* (Hanssen *et al.* 2006). The fact that females with larger wing patches started breeding earlier supports this conclusion, as females arriving earlier at the breeding grounds are likely to be females of higher phenotypic quality (Potti & Montalvo 1991b, Lundberg & Alatalo 1992, Faivre *et al.* 2001). Males might thus obtain an indirect benefit if mated with females showing large wing patches, as ornamented females of high immunological quality would transfer to their offspring either good genes or increased levels of immunoglobulins through the eggs (Saino *et al.* 2001, Morales *et al.* 2006), which is known to be advantageous for the growing chicks (Apanius 1998).

However, we found no evidence that the levels of total immunoglobulins were associated with wing patch size early in breeding in 2004 or with the expression of a forehead patch. If these ornaments functioned as signals of quality, it could be that the strength of the signal content ('parasite resistance') would depend on the relative importance of parasitic infections in different years. Nevertheless, to demonstrate the potential signalling role of these two ornaments, an experimental approach is needed. Also, more data on immune parameters would allow a more complete picture of how these two ornaments are associated with immunocompetence. The absence

of positive correlations between the ornaments and the other health indicators could be interpreted from another point of view. As sexual advertisement is a life-history trait, subject to trade-offs with other traits, ornament expression need not correlate positively with health state (Kokko 2001). Thus, despite the widely held assumption that positive associations between attractiveness and other life-history traits provide evidence for 'good genes' (Møller & Alatalo 1999), positive, neutral or even negative associations are possible under varying circumstances (Gustafsson *et al.* 1995, Kokko 2001, Jones *et al.* 2004), even if the ornament honestly advertises individual quality.

Reproductive performance

The expression of female forehead and wing patches was not associated with female investment in nestling provisioning, contrary to previous studies in other species (Linville *et al.* 1998, Massaro *et al.* 2003; but see Smiseth & Amundsen 2000). Male provisioning rates were not associated with female ornamentation either. Therefore, these results on provisioning effort give little support to the signalling role of female ornamentation in the Pied Flycatcher. However, it could be that 1 h of observation at each nest is a poor estimate of provisioning rate to detect any association with ornamentation. Nevertheless, female ornament expression was associated with some aspects of reproductive success. Forehead patch expression was associated with higher number of nestlings fledged and wing patch size was positively associated with laying date and with the number of hatchlings. If ornamented females are older, the positive associations between female ornamentation and reproductive success could have been interpreted as a consequence of their breeding experience (Potti & Montalvo 1991b, Faivre *et al.* 2001), but female age was not associated with the number of fledglings or hatchlings. Thus, the enhanced reproductive performance shown by ornamented females might not be explained by breeding experience, but by female quality. That females with larger wing patches raised more hatchlings but not more fledglings was not due to a higher nestling mortality. The fact also that male quality greatly affects nestling performance could make it difficult to detect an effect of female quality on the number of fledglings. But an effect was found for the number of hatchlings, which confirms that female quality might be crucial for offspring survival at initial phases (i.e. during incubation or at the beginning of the nestling period).

The expression of female ornaments was not associated with male ornaments within breeding pairs. Only in young birds did we find that wing patch size was marginally and positively associated between mates. In addition, for older females paired with 1-year-old males there was a marginal and positive association between wing patch size of mates. It has been argued that male Pied Flycatchers do not choose females (Dale & Slagsvold 1994). However, no study has experimentally addressed the possibility that males base their courtship on female ornament expression. In the present study, no test for mutual sexual selection has been conducted and, owing to the numerous factors that can affect mating patterns, the possibility of male preference for female white patches remains unresolved.

Another possibility would be that ornament expression was directed towards other female competitors. Ornamented females may thus benefit by showing superior quality during mate choice or nest-site selection and defence. Female aggressive behaviour towards other females for nest-sites and mates has been reported in the Pied Flycatcher (Slagsvold *et al.* 1992, Dale & Slagsvold 1995, Rätti 2000). Thus, future experimental studies, for instance manipulating female ornaments, would help to elucidate if female showiness may affect intra-sexual interactions in this species.

In conclusion, we argue that other proximate factors apart from age determine the expression of female forehead and wing patches in this species. We also give some evidence that these unrelated epigamic traits, little studied in the context of female ornamentation, are associated with some aspects of reproductive success (i.e. number of hatchlings and fledglings), but not with others, such as parental provisioning effort. In addition, female wing patch size was associated with levels of total IgY, a measure of general health status. Further experimental studies could test behaviourally if the receivers of the signal content may be males or other females. Although correlative, this study provides an interesting background for future experimental studies on the adaptive function of female ornamentation.

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